CHAPTER II

ANALYTICAL METHODS FOR GLUCOSE AND FRUCTOSE DETERMINATIONS

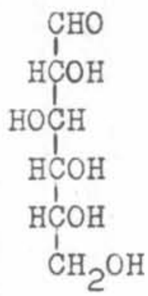
2.1 Introduction

Glucose and fructose are the monosaccharides having 6 carbon atoms and known as hexose (5). They are the structural isomer of each other because of having the same molecular formula ($C_6H_{12}O_6$) but difference structures (6). Glucose contains an aldehyde group and is known as an aldose while fructose contains a keto group and is known as a ketose. An aldo and a keto structure are shown in Figure 2 (6, 7).

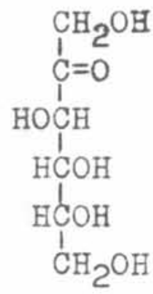
Aldehyde and keto groups in glucose and fructose respectively, can totally reduce alkaline solution of many metallic salts. The quantity of reduced metal thus generated is a measure of total glucose and fructose as total reducing sugars. More selective method is necessary, however, to detemine the content of either glucose or fructose in a mixture of the two sugars.

2.2 Special methods for fructose and glucose determinations

A number of such special quantitative methods for fructose determination are, for example, polarimetric method, diphenylamine method, Wiley method, Jackson - Mathews methods, cysteine carbazole method (8). For glucose determination, special quantitative methods are Iodometric method, Sieben method, glucose oxidase method (8, 9).



D - glucose



D - fructose

<u>Figure 2</u> Aldose and ketose structure (6,7)

Lothrop and Holmes used the Iodometric method to find the apparent glucose (not corrected for the reduction of iodine by fructose) and Munson - Walker method to determine the total reducing sugars calculated as glucose. The approximate percentage of fructose is found by deducting the apparent glucose from the total reducing sugars calculated as glucose. The true glucose is then calculated by deducting from the apparent glucose 0.012 percent of the approximate fructose. Finally the true fructose is found by deducting the true glucose from the total reducing sugars calculated as glucose. The sum of true glucose and true fructose gives the true total reducing sugars (8).

Jackson and Mathews modified Nyns' selective method. They used the Ost solution to find the apparent fructose (glucose has proximately 1/12 reducing power of fructose) and the Lane - Eynon method to find the total reducing sugars calculated as fructose. The true glucose and fructose were then computed by iterative method (10).

2.3 Lane - Eynon method for total reducing sugar determination

It may be sufficient to classify sugar broadly into reducing and non - reducing sugars. All the simple sugars and most reducing disaccharides reduce alkaline solutions of many metallic salts, including those of copper, silver, bismuth and mercury (11). Fehling's solution is the alkaline copper solution and usually used in the reducing sugar determination. The more modern methods which employ Fehling's solution may be divided into two general classes. One is the volumetric method based upon the complete reduction of a measured volume of standard solution and the other is the method in which an excess of standard solution is employed and the reduced copper is then determined gravimetrically or colorimetrically. The example of these two general classes are the Lane-Eynon volumetric method and the Munson - Walker gravimetric method (8).

In Lane - Eynon volumetric method, cuprous oxide is quantitatively precipitated from Fehling' s solution by the reducing action of invert sugar. Methylene blue indicator is reduced to its colorless leuco form in alkaline solution by a slight excess of invert sugar after all the copper has been precipitated, the end point being taken when the blue color of the indicator is completely discharged and the contents of the flask assume the red color due only to cuprous oxide (12).

Fehling's solution consists of two solutions: solution A containing 34.639 gm of crystallized copper sulfate to 500 ml, and solution B containing 173 gm of Rochelle salt (potassium sodium tartrate) and 50 gm of sodium hydroxide to 500 ml. The solutions are to be kept separately until just before using (8). In the mixed Fehling's solution, copper sulfate will react with sodium hydroxide to form copper hydroxide which combines with potassium sodium tartrate to form the complex salt, as indicated by the following equation:

$$CuSO_4 + 2NaOH \longrightarrow Cu(OH)_2 + Na_2SO_4$$

The reducing property of invert sugars is due to their aldehyde or keto groups. The aldehyde group is oxidized by the oxygen withdrawn from the metallic base to the acid carboxyl group, as indicated by the equation:

$$\text{H}-\overset{|}{\text{C}}=\text{O} \quad + \quad 2\text{CuO} \longrightarrow \text{H}-\text{O}-\overset{|}{\text{C}}=\text{O} \quad + \quad \text{Cu}_2\text{O}$$

Aldehyde Copper oxide Acid Cuprous oxide

The extent of the reduction of Fehling's solution varies with the experimental conditions. Of these, the alkalinity of the reagent, the rate and time of heating and the concentration of the sugar in the samples appear to be most important (13). Careful attention to the experimental details is essential if consistent results are to be obtained.

2.4 Iodometric method for apparent glucose determination

During the last few years the Iodometric (Iodimetric) process for the estimation of reducing sugars has been examined by a number of investigators. The process consists essentially of adding a known amount of standard iodine solution to a given quantity of the dilute sugar solution, rendering the mixture alkaline with sodium hydroxide solution and allowing it to stand for a short time, then re-acidifying and titrating the excess of iodine with standard thiosulfate solution (14). The whole series of operations is carried through quite rapidly, and with a minimum of apparatus and reagents which are readily available in any laboratory, so that the process recommends itself admirably for factory analysis.

Most observers find a quantitative oxidation of glucose to gluconic acid by the action of alkaline iodine, according to the equation: (9, 14)

$$C_6H_{12}O_6 + I_2 + 3NaOH \longrightarrow C_5H_{11}O_5COONa + 2NaI + 2H_2O$$

Iodine oxidizes aldoses (e.g. glucose, lactose) but it has little or no effect on ketoses (e.g. sucrose, fructose) , so the former sugars can be determined in the presence of the latter.

The extent of the reduction of alkaline iodine solution varies with the experimental conditions. The alkalinity and the acidity of the reagent, amount of standard iodine solution, the reaction time and the concentration of the sugar in the samples appear to be most important.

2.5 General formula for fructose determination

Lothrop and Holmes's technique using the combination of Munson-Walker method and Iodometric method seemed to give satisfactory determination of fructose. Lane-Eynon method can also be used to determine the total content of reducing sugars. However, Lothrop and Holmes did not derive the value of correction factor accounting for some reduction of iodine by fructose (8). General formula will be formulated to show the relationship among the total content of reducing sugars, apparent content of glucose, true content of fructose and correction factor.

Let G_t = Total content of reducing sugars calculated as glucose in fixed volume of sample solution determined by Lane - Eynon method.

G_I = Apparent content of glucose in fixed volume of sample solution determined by Iodometric method

F_I = Apparent content of fructose in fixed volume of sample solution determined by Iodometric method

G = True content of glucose in fixed volume of sample solution

F = True content of fructose in fixed volume of sample solution

f = Correction factor accounting for some reduction of iodine by fructose

In Lane - Eynon method, G_t may be expressed as:

$$G_t = G + F \qquad (1)$$

In Iodometric method, G_I is expressed as:

$$G_I = G + \frac{F}{f} \qquad (2)$$

F_I may be defined as:

$$F_I = G_t - G_I \qquad (3)$$

The combination of equation (1), (2) and (3) gives the general formula for fructose determination as:

$$F = \frac{(G_t - G_I)}{(1 - \frac{1}{f})}$$

$$\text{or} \quad F = \frac{(G_t - G_I)f}{(f - 1)}$$

The value of f can be determined from model solution of known G and F experimentally.